

Per-/polyfluoroalkyl substance concentrations in human serum and their associations with liver cancer

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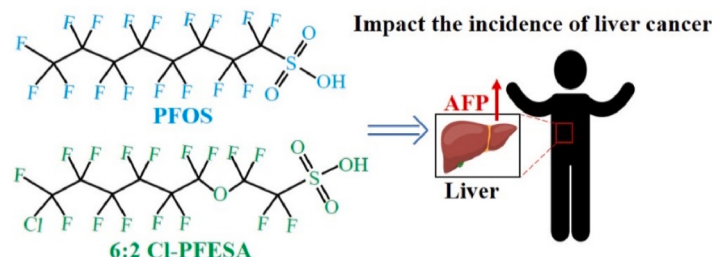
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HIGHLIGHTS

- Occurrence of PFASs in Chinese population serum was investigated.
- PFOS was the most abundant PFAS in human serum from Hangzhou, China.
- PFOS and 6:2 Cl-PFESA levels were positively associated with the levels of AFP.
- PFOS and 6:2 Cl-PFESA levels were correlated with the OR of liver cancer.

GRAPHICAL ABSTRACT



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ABSTRACT

Per-/polyfluoroalkyl substances (PFASs) are widespread in global human blood, and have some toxic effects on liver. However, effects of PFAS exposure on human liver cancer (LC) risk are still not known. In this study, 203 LC patients and 203 controls were recruited, and their serum samples were collected between 2019 and 2021. We determined the residues of 12 PFASs in serum from all participants and quantified their association with LC incidence and tumor markers. PFOS (9.8 ng/mL) had the highest mean concentration in human serum, followed by PFOA (8.3 ng/mL) and 6:2 Cl-PFESA (3.9 ng/mL). We found that concentrations of PFOS and 6:2 Cl-PFESA in human serum were significantly correlated with the levels of alpha fetoprotein (AFP) ($\beta_{\text{PFOS}} = 0.13$, 95% confidence interval (CI)_{PFOS}: 0.088, 0.17; $\beta_{6:2 \text{ Cl-PFESA}} = 0.070$, CI_{6:2 Cl-PFESA}: 0.036, 0.10). A positive association of PFOS and 6:2 Cl-PFESA with odds ratios (OR) of LC (OR_{PFOS} = 0.609, CI_{PFOS}: 1.179, 4.029, $P = 0.001$; OR_{6:2 Cl-PFESA} = 1.844, CI_{6:2 Cl-PFESA}: 1.176, 2.512, $P = 0.02$) were found, after adjusting for different covariates. Moreover, serum PFOA concentrations were associated with carcinoembryonic antigen (CEA), but their correlation with the LC incidence was not statistically significant. This new finding supports the evidence for the positive associations among PFAS exposure, change of specific tumor marker, and LC risks.

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1. Introduction

Perfluoroalkyl substances (PFASs) are a series of organic compounds containing at least one perfluorinated carbon atom, and are widely used as surfactants, non-conductive coolants, water and oil repellents, protective barriers or coatings, surface modifiers, specialty lubricants, and flame retardants, etc (Prevedouros et al., 2006). They have the characteristics of environmental persistence, bioaccumulation, and potential hazards (Castiglioni et al., 2015), and are globally detected in various environmental media. PFASs tend to accumulate in protein-rich parts such as blood and liver, which are different from other persistent organic pollutants that tend to accumulate in lipids (Maestri et al., 2006; Yoo et al., 2009). They have been detected in wildlife (Giesy and Kannan, 2001) and in human blood (Calafat et al., 2006; Midasch et al., 2006).

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), as the final products of degradation of many PFASs and also the two most studied PFASs (Richterova et al., 2018), were widely detected in different environmental media (Mahiba Shoeib et al., 2005) and human blood (Perez et al., 2013). Owing to the high polarity and strong covalent bond between carbon atoms and fluorine atoms, PFOA and PFOS are almost non-biodegradable in the environment (Buck et al., 2011) and are very persistent in organisms. For example, the mean half-lives of PFOA and PFOS in human serum are approximately 3.8 years and 5.4 years, respectively, due to the reabsorption processes involving the liver, intestine, and kidney (Xu et al., 2020). Studies have shown that PFOA and PFOS could be released into the surrounding environment during the production, application and utilization of related products (Liu et al., 2016), so there are multiple routes of human exposure to PFOA and PFOS (e.g., food, drinking water, air, dust, and dermal exposure through direct contact with consumer products and occupational exposure) (Schrenk et al., 2020).

Meanwhile, PFOA and PFOS could disrupt the normal endocrine activities of the human body and cause adverse health effects on humans, including immunosuppression, lower lipoprotein cholesterol density, and lower birth weight (Fisher et al., 2013; Jin et al., 2020; Lindstrom et al., 2011). The CONTAM group study also concluded that potential adverse effects may be observed in humans if they are exposed to PFOS and PFOA above a tolerable weekly intake (TWI, the Health Based Guidance Value) (Schrenk et al., 2020). Based on growing concerns about the potential adverse health effects of PFASs, the main manufacturer, 3 M Company, voluntarily ceased the production of PFOS and compounds that may degrade into PFOS by the end of 2002 (Buck et al., 2011); PFOS has been classified as a kind of persistent organic pollutants (POPs) under the Stockholm Convention since May 2009, PFOA was also listed as a substance of high concern in 2013 (EU, 2019).

Chlorinated polyfluorinated ether sulfonates (Cl-PFESAs), including 6:2 Cl-PFESA and 8:2 Cl-PFESA, are PFOS substitutes exclusively produced in China and are widely used as mist suppressants for the electroplating industry (Pan et al., 2018). Moreover, with the gradual phase-out of PFOS (Wang et al., 2013), Cl-PFESAs entering the environment through industrial wastewater continue to increase. Among them, as the most frequently detected Cl-PFESAs in surface water and sludge samples from China (Jin et al., 2020), 6:2 Cl-PFESA was also detected in animal tissues and human blood, urine, as well as breast milk (Awad et al., 2020; Shi et al., 2015). Some epidemiological studies have observed obvious associations between 6:2 Cl-PFESA and blood pressure (Mi et al., 2020), neonatal glucocorticoid levels (Liu et al., 2020), and cardiac development (Yang et al., 2020). Meanwhile, several in vivo studies have shown that the half-life of 6:2 Cl-PFESA is about 15.3 years (Shi et al., 2016), which suggested that the bioaccumulation of 6:2 Cl-PFESA far exceeds that of legacy PFASs (Mi et al., 2021). Due to these reasons, it is necessary to detect the concentration of Cl-PFESA in the human body and explore its potential toxic effect.

Numerous studies have reported the association between the concentration of perfluoroalkyl substances and liver diseases. An animal study of Sprague Dawley rats showed that the non-neoplastic effects

were in livers of females and males, such as hepatocellular hypertrophy and increased eosinophilic granulation of the cytoplasm, after exposure to potassium PFOS for two years (Butenhoff et al., 2012). A study using zebrafish as a model found liver steatosis in the liver of males after exposure to 0.5 μ M PFOS (Cheng et al., 2016). Previous studies also reported that PFAS exposure is related to increased cancer risks. For instance, a retrospective cohort study reported that high-exposure workers of fluoride production for more than one year had higher risks of bladder cancer (Alexander et al., 2003). As PFAS enter the human body, they are mainly concentrated in the liver, and the liver is an important organ for metabolic activities in the human body (Alexander et al., 2003; Butenhoff et al., 2012; Ojo et al., 2020). However, another study from Italy evaluated the exposure to PFASs in contaminated drinking water and PFASs had a significantly lower RR for liver cancer (RR = 0.84, CI: 0.74, 0.94). Therefore, it is necessary to quantify the levels of PFAS in human specimens and look for the correlations between PFAS exposure and different health outcomes, including liver cancer (LC).

In this study, 406 case-control pairs (203 LC patients and 203 non-cancer people) were recruited in Hangzhou, China during 2019–2021, and whole blood samples were collected. The objectives are to quantify levels of 12 PFASs in serum matrices and to examine the association between serum PFAS levels and tumor markers of LC with various confounding influencing factors using the logistic regression model.

2. Methods and materials

2.1. Population recruitment and sample collection

From January 2019 to May 2021, human serum samples from 203 LC patients and 203 non-cancer people (controls) were obtained from the First Affiliated Hospital of Zhejiang University School of Medicine. With the guidance and help of professional nurses, each participant signed an informed consent form. The demographic characteristics of the participants were reviewed from the medical record system, and complete demographic information was ultimately collected, including height, weight, age, gender, etc. The research protocol was approved by the ethics committee of First Affiliated Hospital of Zhejiang University School of Medicine. The age of studied subjects ranged from 12 to 84. The selection criteria of the research subjects were minimal, namely, 1) being able to communicate in Chinese and complete the questionnaire independently, 2) the control group are healthy individuals, and the case group have no other diseases except liver cancer, such as mental illness, genetic disease, and cardiovascular disease, etc.

Approximately 2–3 mL of serum was collected from each subject under the operation of professional nurses for the measurements of the concentrations of various individual PFAS compounds. The samples were transferred into glass test tubes immediately. All the samples were stored in a -80°C refrigerator before further laboratory analysis of PFASs.

2.2. Sample extraction

A total of 12 PFAS analytes, including seven PFCAs (namely, perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA, Perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnA), and perfluorododecanoic acid (PFDoA)), three PFESAs (namely, perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS) and PFOS), and two Cl-PFESA (namely, 6:2 Cl-PFESA and perfluoro(2-((8-chlorohexyl)oxy)ethanesulfonic acid) (8:2 Cl-PFESA)), were purchased from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC) grade methanol and acetonitrile were purchased from Anpu Experimental Technologies (Shanghai, China). The Milli-Q water used in the experiment was prepared in the laboratory (Academic A10, Germany).

The target PFASs in serum were extracted using ion-pair extraction.

The serum concentrations of PFASs were measured using a liquid chromatograph coupled to a tandem mass spectrometer (Hu et al., 2021; Xu et al., 2020b). Briefly, 0.5 mL of serum was transferred into a 15 mL polypropylene (PP) tube and was fortified with 0.3 ng isotope-labeled standards. The fortified sample was extracted with 8 mL of acetonitrile by vortexing for 2 min and sonicated for 30 min. After centrifugation at 4000 rpm for 10 min, the supernatant was transferred to a new PP tube. The extraction was performed once more, adding 4 mL of acetonitrile, and combining it into that new PP tube. The combined supernatants were dried under gentle nitrogen gas and reconstituted with 100 μ L of 1:1 methanol-water solution, followed by vortexing for 1 min and centrifugation at 9960 rpm for 6 min. The sample was transferred into a 1.5 mL autosampler vial and was stored in a -20°C refrigerator before instrumental analysis.

2.3. Quality assurance and quality control

Solvent blank analysis using HPLC grade methanol was performed for each batch of 5 samples to monitor instrument contamination. One Milli-Q water was inserted in each batch of 10 samples as a procedural blank sample to monitor background contamination. In this study, no PFAS was found in the procedure blank samples, except for PFOA. The mean concentration of PFOA detected in the procedure blank plus 10-fold standard deviation was used as the limit of quantification (LOQ) for PFOA (0.09 ng/mL) in this experiment, while the LOQs for the remaining PFAS were calculated using 10-fold signal-to-noise values ranging from 0.07 to 0.10 ng/mL (see Table S2). We used the internal standard method to quantify 12 PFAS in human serum. Six concentrations were set for the standard series of working solutions, including 0.1, 0.5, 1, 5, 10, and 20 ng/mL. Human serum ($n = 3$) and fetal bovine serum ($n = 1$) were spiked with a mixed standard sample of 0.1 ng/mL (LOQ concentration) and 1 ng/mL (standardized midpoint concentration) to calculate spiked recoveries. Spiked recoveries range from 72.6% to 131%. To ensure the exact quantification of PFAS, a US NIST standard (SRM1957; NIST) was used as a quality assurance sample. The relative uncertainty was in the acceptable range (3.89%–9.78%) by comparison with the standard values.

2.4. Statistical analysis

We performed preliminary descriptive statistics on subjects' demographic information, tumor marker parameters, and serum PFAS concentration levels. Spearman correlation analysis (β) was used to assess the association between individual serum PFAS concentrations and serum tumor marker levels. Linear regression analysis was used to assess serum PFAS concentrations with tumor markers of LC which was based on the stepwise method to achieve the optimal model. All subjects' gender (male and female), age (<30, 30–40, 41–50, 51–60, and >60), education level (>12, 9–12, and <9), BMI, and annual household income (<50,000, 50,000–100,000, and >100,000) were ultimately included in the optimized model. Logistic regression analysis was used to determine the association between serum PFAS concentrations and the incidence of LC after adjusting different covariates. To achieve a normal distribution of the residuals, the independent variable (PFAS concentrations) and the dependent variable, including Alpha fetoprotein (AFP), Carcinoembryonic antigen (CEA), Carbohydrate antigen 12-5 (CA12-5), Carbohydrate antigen 19-9 (CA19-9) were all log-transformed. The assumption of normality of the residual regression plots was confirmed by visual inspection. For the samples with concentration below LOQ, we replaced them with the level of $\text{LOQ}/\sqrt{2}$. All statistical analyses were conducted using IBM SPSS Statistics software (version 25; Chicago, IL, USA) with significance set at $p < 0.05$.

3. Results

3.1. Demographic characteristics

Tumor markers (mean $\pm$ standard deviation (SD)) (including alpha fetoprotein (AFP); carcinoembryonic antigen (CEA); carbohydrate antigen 12-5 (CA 12-5); carbohydrate antigen 19-9 (CA19-9)) and demographic characteristics data of all participants [n (%)] are shown in Table 1. In this study, the BMI of the studied population was divided into 5 intervals of ≤ 18.4 , 18.5–23.9, 24.0–27.9, as well as ≥ 28.0 , and more than 50% population of BMI in this paper between 18.5 and 23.9. Overall, no significant difference was observed in the demographic information characteristics between the case and control groups.

3.2. Concentrations of PFASs in human serum

The concentration levels of PFASs in human serum are presented in Table 2. The total concentrations of PFASs ($\sum$ PFASs) ranged from 6.6 to 250 and 4.3–87 ng/mL in the case and control groups, respectively. Among PFCAs, PFOA and PFNA had 100% detection frequencies in all subjects. For PFASs, the detected frequencies of PFBS, PFHxS, and PFOS were all 100%. Moreover, 6:2 Cl-PFESA was detected in all samples, while its by-product 8:2 Cl-PFESA was detected in 48% and 58% of the case and control groups, respectively. PFOS had the highest concentration in all human serum samples (mean: 9.8 ng/mL, range: 0.71–52 ng/mL), accounting for more than 30% of the total PFAS concentration (Fig. 1), followed by PFOA (8.3 ng/mL, 0.67–87 ng/mL) and 6:2 Cl-PFESA (3.9 ng/mL, 0.07–57 ng/mL) (Table 2). The concentrations of C_9 – C_{12} PFCAs (0.13–0.49 ng/mL) were comparable in the case and control groups. The concentrations of 8:2 Cl-PFESA (<LOQ, <LOQ–22 ng/mL) and PFHpA (<LOQ, <LOQ–3.2 ng/mL) measured in human serum were relatively low in the case group. The serum concentrations of PFOA, PFOS, and 6:2 Cl-PFESA were significantly higher in the case group than those in the control group ($p < 0.05$).

Table 1
Demographic characteristics and tumor markers of liver cancer in cases and controls ($n = 406$; n (%) or mean $\pm$ SD).

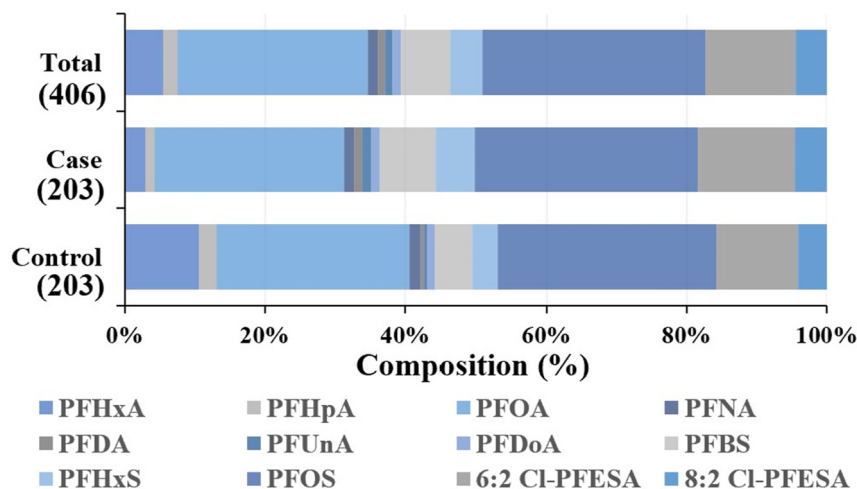
Characteristics	Case ($n = 203$) n (%) or mean $\pm$ SD	Control ($n = 203$) n (%) or mean $\pm$ SD
Sex		
Male	127 (62.6)	128 (63.1)
Female	76 (37.4)	75 (36.9)
Age, years		
<30	8 (3.9)	6 (3.4)
30–40	14 (6.9)	12 (6.8)
41–50	34 (16.7)	26 (14.7)
51–60	66 (32.5)	61 (34.5)
>60	81 (40.0)	72 (40.6)
BMI, kg/m^2		
≤ 18.4	9 (4.4)	14 (7.9)
18.5–23.9	122 (60.1)	99 (55.9)
24.0–27.9	58 (28.6)	50 (28.3)
≥ 28.0	14 (6.9)	14 (7.9)
Education level		
<9	78 (38.4)	63 (31.0)
9–12	72 (35.5)	86 (42.4)
>12	53 (26.1)	54 (26.6)
Annual household income		
<50,000	66 (32.5)	59 (29.1)
50,000–100,000	94 (46.3)	81 (39.9)
>100,000	43 (21.2)	63 (31.0)
AFP, ng/mL	856.93 $\pm$ 3956.48	15.17 $\pm$ 28.95
CEA, ng/mL	9.27 $\pm$ 48.90	4.17 $\pm$ 3.89
CA12-5, U/mL	19.48 $\pm$ 15.89	21.21 $\pm$ 14.51
CA19-9, U/mL	95.05 $\pm$ 878.37	300.94 $\pm$ 1138.36

Note: Body mass index (BMI , kg/m^2) = weight (kg)/height² (m^2); AFP, Alpha fetoprotein; CEA, Carcinoembryonic antigen; CA12-5, Carbohydrate antigen 12-5; CA19-9, Carbohydrate antigen 19-9.

Table 2

Distribution of 12 PFAS concentrations (ng/mL) in human serum from liver cancer in case and control groups.

Analyte	DF (%)	Median	Min	Mean	Max	25th	50th	75th	95th
<i>Case (n = 203)</i>									
Perfluorocarboxylic acids									
PFHxA	74	0.81	<LOQ	1.0	2.9	<LOQ	0.81	1.3	2.3
PFHpA	35	0.24	<LOQ	<LOQ	3.2	<LOQ	<LOQ	0.57	1.6
PFOA	100	6.6	0.67	9.3	87	4.5	6.6	11	23
PFNA	100	0.35	0.1	0.49	5.6	0.22	0.35	0.61	1.3
PFDA	100	0.26	0.11	0.37	1.0	0.16	0.26	0.48	0.94
PFUnA	100	0.23	0.1	0.46	14	0.15	0.23	0.51	0.92
PFDoA	59	0.30	<LOQ	0.44	2.9	<LOQ	0.30	0.59	0.97
Perfluoroalkyl sulfonate									
PFBS	100	0.98	0.11	2.7	51	0.42	0.98	2.8	9.2
PFHxS	100	0.85	0.11	1.8	25	0.38	0.85	1.9	6.7
PFOS	100	7.2	0.81	11	52	3.8	7.7	15	34
Chlorinated polyfluorinated ether sulfonate									
6:2 Cl-PFESA	100	2.1	0.14	4.7	57	1.2	2.1	5.8	15
8:2 Cl-PFESA	48	0.88	<LOQ	<LOQ	22	<LOQ	<LOQ	1.8	5.0
Sum of poly- and perfluoroalkyl substances									
ΣPFASs	85	25	6.6	33	250	15	25	42	74
<i>Control (n = 203)</i>									
Perfluorocarboxylic acids									
PFHxA	56	2.2	<LOQ	2.8	13	<LOQ	2.2	3.6	7.1
PFHpA	61	0.34	<LOQ	0.69	5.5	<LOQ	0.34	0.89	3.1
PFOA	100	5.4	0.84	7.3	41	3.75	5.4	8.53	20
PFNA	100	0.32	0.03	0.41	1.8	0.18	0.32	0.57	1.0
PFDA	73	0.11	<LOQ	0.14	0.72	<LOQ	0.11	0.17	0.32
PFUnA	53	0.099	<LOQ	0.13	0.6	<LOQ	0.099	0.16	0.38
PFDoA	48	0.22	<LOQ	0.28	1.3	<LOQ	<LOQ	0.37	0.68
Perfluoroalkyl sulfonate									
PFBS	100	0.38	0.013	1.4	19	0.13	0.38	1.4	6.4
PFHxS	100	0.39	0.081	0.95	7.2	0.17	0.39	1.2	4.2
PFOS	100	5.5	0.71	8.3	36	3.0	5.5	11	28
Chlorinated polyfluorinated ether sulfonate									
6:2 Cl-PFESA	100	1.4	0.07	3.1	21	0.55	1.4	3.3	14
8:2 Cl-PFESA	58	0.79	<LOQ	1.1	9.2	<LOQ	0.79	1.3	3.2
Sum of poly- and perfluoroalkyl substances									
ΣPFASs	79	20	4.3	24	87	12	20	30	64

**Fig. 1.** Composition profiles of PFASs in human serum from liver cancer cases and controls ($n = 406$) in Hangzhou.

3.3. Associations of serum PFASs with tumor markers and incidence of liver cancer

Correlations among various individual PFAS concentrations in human serum and associations between human serum concentrations of PFASs and tumor marker levels are all listed in Table S3. The result of Spearman correlation analysis showed a significant correlation between the concentration of C_4 – C_8 PFASs and 6:2 Cl-PFESA ($\beta = 0.45$ – 0.78 , $p < 0.05$) in human serum samples. The human serum concentration of PFOA was correlated with C_4 – C_6 PFASs ($\beta = 0.47$ – 0.63 , $p < 0.05$) but

not significantly correlated with PFOS and 6:2 Cl-PFESA ($\beta = 0.25$ – 0.30 , $p > 0.05$). We found that the concentrations of PFOS and 6:2 Cl-PFESA in human serum were positively correlated with the level of AFP ($\beta = 0.48$ – 0.55 , $p < 0.05$). A positive correlation between PFOA concentration levels and CEA levels ($\beta = 0.40$, $p < 0.05$) were also observed. However, PFOA was not statistically correlated with the levels of other tumor markers ($\beta = 0.021$ – 0.32 , $p > 0.05$).

The coefficients of log-transformed PFOS and 6:2 Cl-PFESA to the dependent variable AFP (PFOS $\beta_{\text{adjusted}} = 0.13$, CI: 0.088–0.17, $p < 0.05$; 6:2 Cl-PFESA $\beta_{\text{adjusted}} = 0.070$, CI: 0.036–0.10, $p < 0.05$) were positively

and statistically significant after adjusting for age, education level, BMI, annual household income, sex, smoking habit, parity, and medical history (Table 3). For PFOA, the slope for CEA levels ($\beta_{\text{crude}} = 0.038$, 95% CI: 0.019, 0.057, $p < 0.05$; $\beta_{\text{adjusted}} = 0.028$, 95% CI: 0.007, 0.051, $p < 0.05$) in both crude and adjusted models were positive and achieved statistical significance. Smoking habit had the greatest impact on changing the adjusted results as compared to the crude ones ($p = 0.02$). The quartiles of serum PFASs concentrations to tumor markers are listed in Table 3. Higher levels of PFOS and 6:2 Cl-PFESA with elevated AFP were observed. PFAS exposure was not significantly associated with the exposure of CA12-5 and CA19-9 ($p > 0.05$) (Table S3).

There were significant associations of serum PFAS concentration with an incidence of liver cancer here (Table 4). The crude odds ratio (OR) for LC was 2.636 for a unit increase in serum PFOS concentration (95% CI: 1.211–4.061) and the adjusted results were statistically significant and similar in magnitude ($\text{OR}_{\text{adjusted}} = 2.609$, CI: 1.179–4.029). For 6:2 Cl-PFESA, the OR was significant in crude and adjusted models with 1.859 (95% CI: 1.198–2.520) and 1.844 (CI: 1.176–2.512), respectively. We did not find a statistically significant association between other individual PFASs and the incidence of LC.

4. Discussion

PFOS has the highest serum concentration among different PFASs in this study, with a detection frequency of 100%. Yeung et al. previously quantified the serum levels of PFOS in Australians with hepatocellular carcinoma (HCC) disease (Yeung et al., 2013). The mean concentration of PFOS in HCC patients ($n = 24$) and controls ($n = 25$) were estimated to be 13.3 and 8.48 ng/mL, which were comparable with those in the present study (11 and 8.3 ng/mL, respectively). Compared with previous studies, the mean serum PFOS concentration detected in LC cases in this study was much higher than that in Taiwanese breast cancer patients (5.64 ng/mL) (Tsai et al., 2020). These suggest that the population in Hangzhou, China was exposed to substantial PFOS. PFBS and PFHxS were detected in 100% of all serum samples from the population participating in this study, with respective mean concentrations of 2.1 ng/mL and 1.4 ng/mL. Mean serum levels of PFHxS in Hangzhou were similar to those of males (1.22 ng/mL) in Poland (Goralczyk et al., 2015) but higher than those of German adolescents (0.54 ng/mL) (Duffek et al., 2020). This may be related to the more frequent exposure of the population from southeast China to short-chain PFASs.

For PFCAs, PFOA is detected in all serum samples, but PFHxA,

Table 4

Evaluated odds ratios (OR, 95% CI) in liver cancer associated with each incremental value of log-transformed PFAS concentrations in human serum ($n = 406$) using logistic regression model.

	Crude		Adjusted	
	OR (95% CI)	P_{trend}	OR (95% CI)	P_{trend}
PFOA	1.040 (1.005, 1.075)	0.06	1.036 (1.002, 1.070)	0.07
PFNA	1.276 (0.883, 1.669)	0.77	1.269 (0.876, 1.662)	0.11
PFDA	1.192 (0.965, 1.419)	0.3	1.184 (0.964, 1.404)	0.46
PFBS	1.138 (1.037, 1.239)	0.09	1.129 (1.028, 1.230)	0.6
PFHxS	1.182 (0.884, 1.480)	0.2	1.191 (0.903, 1.479)	0.1
PFOS	2.636 (1.211, 4.061)	0.01	2.609 (1.179, 4.029)	0.001
6:2 Cl-PFESA	1.859 (1.198, 2.520)	0.03	1.844 (1.176, 2.512)	0.02

PFHpA, and PFDoA were detected in only 65%, 46%, and 53% of the serum samples. The mean serum level of PFOA (8.3 ng/mL) in Hangzhou is higher than the data from previous studies. For example, the mean concentrations of PFOA in Jeddah (Banjabi et al., 2020), Arvidsjaur (Xu et al., 2020a), Kharkiv (Ludwicki et al., 2015), and USA (Geiger et al., 2014) were 1.7 ng/mL, 6.5 ng/mL, 1.8 ng/mL, and 4.2 ng/mL, respectively. This hints at a possible higher exposure to PFOA in the Hangzhou population, or possibly because PFOA has a long half-life in humans and tends to lead to enrichment in the body (Olsen et al., 2007). Here, the mean levels of C₉–C₁₂ PFCAs in human serum, including PFNA (0.45 ng/mL), PFUnA (0.35 ng/mL), and PFDoA (0.37 ng/mL) are almost the same. In addition, the mean human serum level of PFNA here is lower than that in Vancouver (0.59 ng/mL) (Makey et al., 2017), Sri Lanka (0.47 ng/mL) (Guruge et al., 2005), Aarhus (0.76 ng/mL) (Bjerregaard-Olesen et al., 2016), but higher than that in New Jersey (0.35 ng/mL) (Graber et al., 2019), and Guangzhou (0.23 ng/mL) (Zhang et al., 2017).

6:2 Cl-PFESA dominated Cl-PFESAs with a contribution of 12.8% in all detected PFASs (mean concentration: 3.9 ng/mL). 8:2 Cl-PFESA was detected in 53% of the serum samples with a mean concentration of 1.3 ng/mL. It is not surprising that 6:2 Cl-PFESA is prevalent in human serum, with reports indicating an annual production of 20–30 tons of 6:2 Cl-PFESA in China, and other studies reporting the prevalence of 6:2 Cl-PFESA in marine organisms, agricultural soils, and the breast milk of Chinese pregnant women (Awad et al., 2020; Chen et al., 2018; Lan et al., 2020).

Concentrations of PFOS in serum were found to be statistically correlated with AFP, which is a glycoprotein and an important tumor

Table 3

Linear regression coefficients β (95% Confidence Interval) for tumor markers and log-transformed PFAS concentrations in LC patients ($n = 203$) and control group ($n = 203$).

Analyte	AFP		CEA		CA12-5		CA19-9	
	Crude β (95% CI)	Adjusted β (95% CI)	Crude β (95% CI)	Adjusted β (95% CI)	Crude β (95% CI)	Adjusted β (95% CI)	Crude β (95% CI)	Adjusted β (95% CI)
PFOA	−0.011 (−0.033, 0.011)	0.063 (−0.082, 0.14)	0.038 (0.019, 0.057)	0.028 (0.007, 0.051)	0.009 (0.002, 0.016)	0.076 (−0.045, 0.12)	0.022 (0.004, 0.04)	0.019 (−0.099, 0.080)
PFNA	−0.15 (−0.56, 0.26)	−0.038 (−0.29, 0.25)	0.12 (−0.058, 0.300)	0.007 (−0.70, 0.71)	0.087 (−0.045, 0.21)	0.053 (−0.61, 0.67)	0.034 (−0.31, 0.38)	0.005 (−0.25, 0.25)
PFDA	0.28 (−0.56, 1.13)	−0.017 (−0.44, 0.42)	0.15 (−0.19, 0.35)	0.025 (−0.23, 0.25)	−0.19 (−0.45, 0.063)	−0.034 (−0.29, 0.25)	−0.77 (−1.41, −0.64)	−0.073 (−0.12, 0.049)
PFBS	−0.016 (−0.058, 0.026)	−0.017 (−0.14, 0.12)	0.01 (−0.008, 0.028)	0.067 (−0.28, 0.41)	0.005 (−0.008, 0.018)	0.013 (−0.30, 0.33)	−0.017 (−0.051, 0.034)	−0.031 (−0.23, 0.20)
PFHxS	−0.007 (−0.082, 0.068)	−0.007 (−0.22, 0.22)	0.003 (−0.033, 0.030)	0.008 (−1.35, 1.59)	0.014 (−0.010, 0.024)	0.013 (−0.54, 0.52)	−0.024 (−0.086, 0.062)	−0.039 (−0.17, 0.13)
PFOS	0.10 (0.083, 0.11)	0.13 (0.088, 0.17)	0.03 (−0.098, 0.16)	0.02 (−0.095, 0.13)	0.012 (−0.006, 0.030)	−0.017 (−0.19, 0.13)	0.016 (0.004, 0.028)	−0.021 (−0.16, 0.14)
6:2 Cl-PFESA	0.077 (0.043, 0.11)	0.070 (0.036, 0.10)	0.08 (−0.11, 0.27)	0.09 (−0.098, 0.28)	0.081 (0.053, 0.13)	0.038 (−0.160, 0.12)	0.003 (−0.022, 0.028)	0.005 (−0.020, 0.030)

Note: Models adjusted for age, education level, BMI, annual household income, sex, smoking habit, medical history. CI, confidence interval. AFP, Alpha-fetoprotein; CEA, Carcinoembryonic antigen; CA12-5, Carbohydrate antigen 12-5; CA19-9, Carbohydrate antigen 19-9; PFBS, perfluorobutane sulfonate; PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; PFNA, perfluorononanoate; PFDA, perfluorodecanoate; Cl-PFESA, chlorinated polyfluorinated ether sulfonate. The numbers in bold font suggest the statistical significance at $p < 0.05$.

marker for the diagnosis of primary LC (Chen et al., 2020; Everman et al., 2000). In addition, the significantly positive association of PFOS concentration in human serum with the incidence of LC was quantified. An *in-vitro* experiment in transgenic zebrafish reported that co-exposure with 500 µg/mL PFOS and 20 mg/L doxycyclines resulted in carcinogenic hepatomegaly and increased the proportion of liver cancer in zebrafish by affecting metabolic pathways (Zhu et al., 2021). Disturbed metabolic pathways mean that tumor cells can be metabolically reprogrammed to obtain energy for cell survival in the absence of nutrition and inadequate stress conditions (Boroughs and DeBerardinis, 2015), also because of abnormal expression of proteins involved in lipid metabolism and exogenous metabolism (Li et al., 2021). Many studies have shown that PFOS exposure causes hepatic steatosis in animals. For example, Marques et al. (2020) pointed out that PFOS administration resulted in increased liver weight and hepatic steatosis in mice, which may be due to the increased uptake of fatty acids (FA) by PFOS-treated hepatocytes (Cheng et al., 2016). Phospholipids, as crucial structural molecules of cell membranes, contain abundant FA, which is needed by tumor cells to proliferate and eventually lead to hepatocarcinogenesis (Zaidi et al., 2013). Moreover, Wiesel et al. (2015) reported that PFOS exposure to hepatocellular carcinoma (HepG2) in the concentration range of 2×10^{-7} to 2×10^{-5} M showed a dose-dependent increase in DNA damage and resulted in a significant increase in ROS production. Another *in-vitro* experiment showed that the strand breaks and the formation of formamidopirimidin-DNA glycosylase-sensitive sites induce DNA damage in human HepG2 cells (Eriksen et al., 2010). A study in rats by continuous gavage for 30 days demonstrated that PFOS exposure induced oxidative DNA damage and apoptosis in rat liver (Eke et al., 2017). Considering that oxidative DNA damage can be mutagenic and carcinogenic, it is plausible that PFOS exposure induces DNA damage making the risk of hepatocellular carcinoma increase. In another animal study, a statistically significant increase in hepatocellular adenomas was observed in male and female rats exposed to PFOS (20 ppm) via dietary exposure, by activating the pathway of PPAR- α (Butenhoff et al., 2012).

Notably, a significant association was discovered between serum 6:2 Cl-PFESA levels and the level of AFP as well as the incidence of LC. There are many studies reporting associations between 6:2 Cl-PFESA and adverse liver outcomes. For example, Zhang et al. (2018) demonstrated that lipid accumulation in the liver and relative weight of the liver was significantly increased in male rats after 56 days of exposure with 1 mg/kg/d 6:2 Cl-PFESA. In a toxicological experiment, 6:2 Cl-PFESA exhibited the disruption of hepatic lipid metabolism and induced hepatomegaly in adult mice, which may be related to the preferential accumulation of 6:2 Cl-PFESA in the mouse liver (Pan et al., 2021). It can conclude that from the results that the main target organ for 6:2 Cl-PFESA is supposed to be liver. More toxicological information is needed in the future to demonstrate the risk of 6:2 Cl-PFESA on LC. Furthermore, serum PFOA concentrations were significantly correlated with CEA but were not statistically correlated with the incidence of LC. CEA is a carcinoembryonic antigen, a broad-spectrum tumor marker that is significantly elevated in many malignancies (Song et al., 2021). Previously, Girardi and Merler studied the association between occupational exposure to PFOA and PFOS and mortality in 120 Italian workers, and showed that serum PFOA concentrations were significantly associated with mortality caused by liver cancer (RR: 6.69, CI: 1.71, 26.2) (Girardi and Merler, 2019). All suggest that the appropriate tumor markers, such as AFP and CEA, can reflect the adverse impacts from exposure of identified PFAS species on the risk for LC.

This study has some limitations. The width of the confidence interval and statistical uncertainty in this study may be increased because of the limited sample size. In addition, LC may be caused by long-term PFAS exposure, but only a single measurement of PFAS was performed in this experiment; although PFAS has a long half-life in humans, historical exposure data from study subjects are still lacking.

5. Conclusions

In this study, PFAS exposure was measured in 406 randomly recruited subjects, including 203 cases and 203 controls. Meanwhile, we quantified the associations between serum PFAS concentrations and tumor markers as well as the incidence of LC. PFOS was the predominant PFASs in human serum, followed by PFOA and 6:2 Cl-PFESA. It was found that increased serum PFOS and 6:2 Cl-PFESA levels are correlated with increased AFP and CEA levels. We also observed that PFOS and 6:2 Cl-PFESA concentrations in human serum were significantly associated with the incidence of LC, demonstrating the evidence that exposure to PFASs can lead to a higher risk of LC.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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